# EX VIVO MR MICROSCOPIC IMAGING IDENTIFIES MULTIPLE NEUROANATOMICAL CORRELATES OF FUNCTIONAL MOTOR DEFICITS IN A RAT MODEL OF BILIRUBIN ENCEPHALOPATHY

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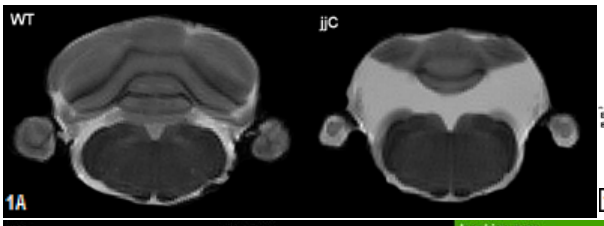
**Target audience:** Neuroscientist, radiologist, neurologists, MR scientists with interest in preclinical models.

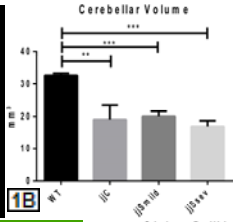
**Purpose**: Bilirubin encephalopathy can either present in the newborn as Kernicterus[1], a devastating disorder of the globus pallidus resulting in dystonic cerebral palsy, or as a chronic encephalopathy preferentially involving the cerebellum in patients with Crigler Najjar syndrome[2], a genetic disorder due to recessive mutations in UDP-glucuronosyltransferase 1A (UDPG1A). The UDPG1A mutant Gunn rat has served as a model of bilirubin encephalopathy[3,4,5]. Our group has performed an in vivo diffusion tensor imaging (DTI) study of the Gunn rat in the acute phase of this condition before. We could show global white matter and thalamic FA changes in acutely dystonic animals as well as profound cerebellar atrophy in all jaundiced animals at postnatal day 17 (P17). The purpose of this current study was to evaluate chronic effects of bilirubin encephalopathy by means of ex vivo DTI and correlate findings to the severity of neurological disability in order to determine whether the Gunn rat can serve as a valid model for bilirubin encephalopathy and secondary dystonia from an MRI point of view, as well as to further enhance our current understanding of these conditions.

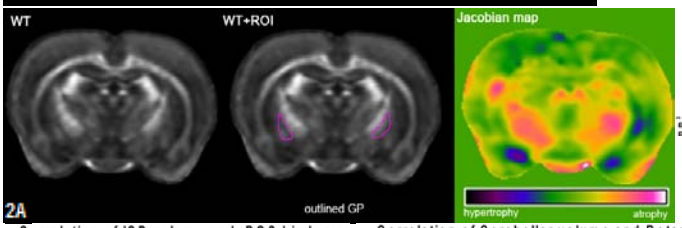
**Methods**: Jaundiced Gunn rats were either treated with saline (jjC, n=4) or with 80mg/kg intraperitoneal Sulfadimethoxine at P15 and compared to wild type controls (WT, n=6). Sulfadimethoxine displaces unconjugated bilirubin manifesting in either severe dystonia (jjSsev n=4) or a less affected phenotype (jjSmild, n=8). Behavioral testing (Catwalk, Rotarod, BBB) was performed at P60 to assess the severity of the dystonic phenotype[6]. A Bruker horizontal 11.7T scanner was used to acquire ex vivo high-resolution T2-weighted and diffusion tensor images in the chronic phase (12-18 weeks). Experiments were carried out with a 25 mm diameter quadrature volume coil. T2-weighted images were acquired using a RARE sequence (TE/TR = 40/3000ms, RARE factor = 8, 0.125 x 0.125 x 0.4 $mm^3$). DTI Data were acquired using a 3D diffusion-weighted EPI sequence with the following parameters: TE/TR = 27.5/600 ms, 30 diffusion directions, b = 1500 $s/mm^2$, and a resolution of 0.2 x 0.2 x 0.2 $mm^3$. Total scan time was 4 hours. Large deformation diffeomorphic metric mapping (LDDMM) was performed based on diffusion weighted images and fractional anisotropy (FA) maps. Jacobian maps were obtained to evaluate volume changes in globus pallidus (GP), middle cerebellar peduncle (MCP), inferior cerebellar peduncle (ICP) and cerebellum by means of region of interest (ROI) analysis in consecutive slices in FA maps and T2-weighted images.

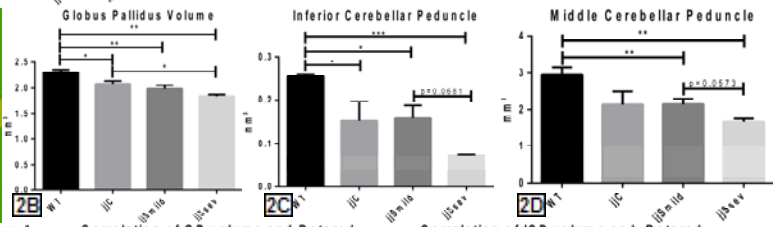
**Results**: All animals in the Sulfadimethoxine-treated group developed ataxia with either mild or severe dystonia, whereas animals in the jj-C group exhibited ataxia without dystonia. While there were no significant changes in FA, cerebellar volume analysis showed highly significant decreases in jaundiced animals compared to WT controls. No significant difference in cerebellar volume was found between jj-C and dystonic animals (Fig. 1B). The GP volume was both significantly decreased in all jaundiced animals compared to the WT and the saline-injected control group compared to the severely dystonic animals (Fig. 2B). Both the ICP and MCP volume showed a significant decrease in volume in all dystonic compared to WT animals as well as a trend towards significant volume differences in the severely dystonic compared to the mildly impaired animals(Fig. 2C-D). Base of support (BOS) of the hind paws, a value obtained in behavioral testing that was significantly increased in severely dystonic compared to WT animals, correlated with the volume of ICP (Fig. 3A). Fall latency on the Rotarod system was significantly decreased in all jaundiced animals and showed correlation to the cerebellar, GP and ICP volumes (Fig. 3B-D).

**Discussion**: While this is a pilot study, our data implicates interesting findings. FA changes occur in the acute phase of bilirubin encephalopathy but are not apparent in the chronic phase. All rats homozygous for the mutation of the UDPG1A gene show highly significantly decreased cerebellar volumes, which is also reported in patients with Crigler-Najjar syndrome. In the chronic phase of bilirubin encephalopathy the GP volume was decreased, confirming in conjunction with behavioral testing data that the Gunn rat may serve as a valid rodent model for secondary dystonia. Interestingly, ICP and MCP show a trend towards volume changes in mildly compared to severely dystonic animals, suggesting an involvement in the development of dystonia. This supports our postulate that cerebellothalamocortical pathways may indeed be a signature of dystonia in rodents in general.



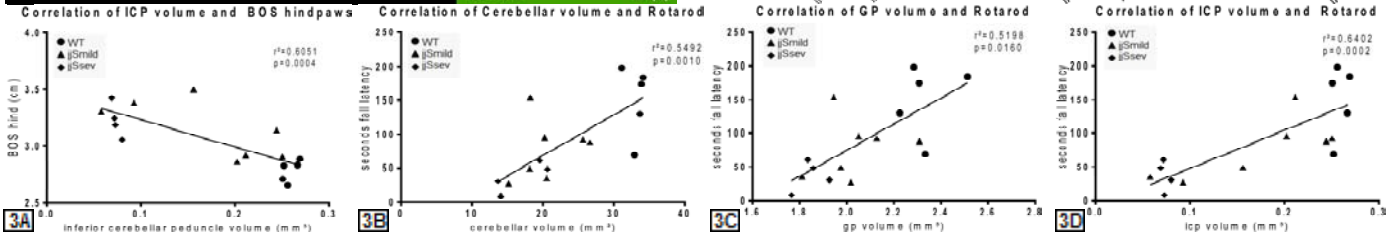




*Fig.1: A) T2-weighted cerebellar cross section; B) Cerebellar volume comparison (**≤0.01; ***≤ 0.0001)*
*Fig.2: A) DTI cross sections of WT and WT showing outlined GP, Jacobian map showing volume differences in jjSsev compared to WT; B-D) Volume comparison of GP, ICP and MCP (*≤0.05)*
*Fig.3: A) Correlation of ICP volume and base of support of hindpaws; B-D) Correlation of Cerebellar, GP and ICP volume and fall latency on Rotarod system*

**References**: **1)** Shapiro, Pediatr Neurol 29 (2003): 410-421. **2)** Tabarki, Pediatr Neurol. 2002 Sep;27(3): 234-6. **3)** Gunn, J Hered 29 (1938): 137-139. **4)** Johnson, Am J Dis Child 101 (1961): 322-349. **5)** Baron, Front Syst Neurosci. 2011;5: 67, **6)** Jinnah, Mov Disord (2005);20(3):283-92